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(71) Applicant *(for all designated States except US)*: CANCER RESEARCH TECHNOLOGY LTD. [GB/GB]; 2 Carlton House Terrace, London SW1Y 5AR (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)* : NEIDLE, Stephen [GB/GB]; JENKINS, Terence, Charles [GB/GB]; AGBANDJE, Mavis [GB/GB]; The Institute of Cancer Research, Block F, Cotswold Road, Sutton, Surrey SM2 5NG (GB).

(74) Agents: GOLDIN, Douglas, Michael et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

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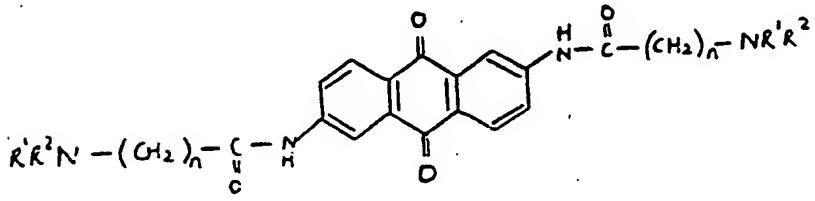


(57) Abstract

Compounds are disclosed having general formula (I), in which n is 1, 2 or 3; and R^1 and R^2 are each, independently, an ethyl, hydroxyethyl, or hydroxymethyl group; or R^1 and R^2, together with the nitrogen atom to which they are attached, form a cyclic group which is a 1-piperidino, 2- or 4-(2-hydroxyethyl)-1-piperidin , 2-hydroxymethyl-1-piperidin , 4-(2-hydroxyethyl)- r 4-methyl-1-piperazino, r 4-morph lino group; or a pharmaceutically acceptable salt thereof. The compounds have utility as anti-cancer agents. A meth d f preparing the compounds, and pharmaceutical compositions containing the compounds, are als disclosed.

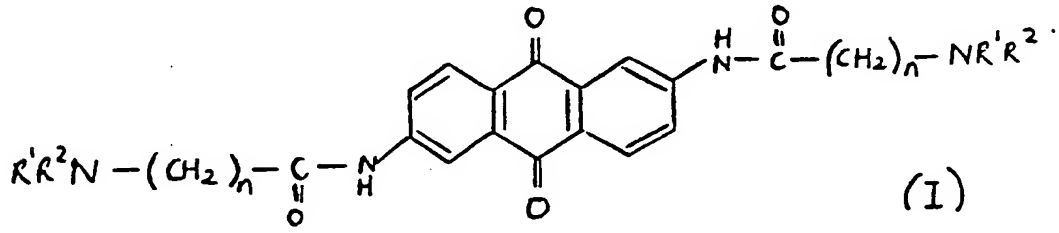
* See back f page

INTERCALATING AGENTS

The present invention relates to anthraquinone derivatives and to their use as intercalating agents.

The search for new anticancer agents is an evolving process and there is a need to develop drugs which show selective activity towards cancer cells. This requires an understanding of the biochemical and genetic basis of the origin of the disease as well as an understanding of the fundamental biochemical differences between cancer and normal cells. Developments in this area, and the increasing understanding of the biochemical activity of cytotoxic drugs, have led to attempts to design novel agents which interact with defined cellular targets.

The present inventors have now developed a series of novel compounds based on anthraquinone which are potential anti-cancer agents. The compounds are capable of binding to DNA, are cytotoxic and non-mutagenic. Accordingly, the present invention provides a compound having the general formula (I):



(I)

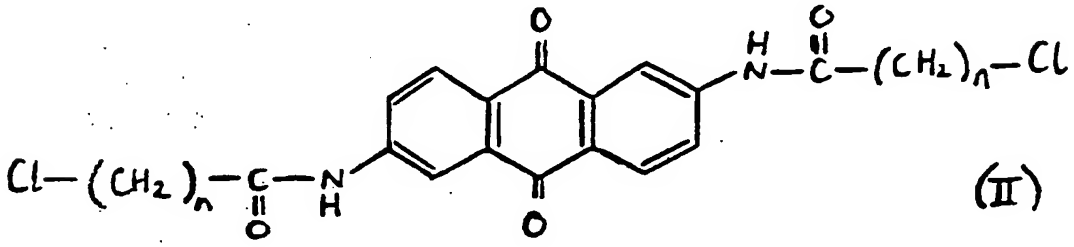
in which:

n is 1, 2 or 3 and R^1 and R^2, which may be the same or different, are each a hydroxyethyl or hydroxymethyl group; or R^1 and R^2 form, with the nitrogen atom to which they are attached, a cyclic group which is a 2-, or 4-(2-hydroxyethyl)-1-piperidino, 1-piperidino, 2-hydroxymethyl-1-piperidino, 4-(2-hydroxyethyl) or 4-methyl-1-piperazino or 4-morpholino group; and pharmaceutically acceptable salts thereof.

A further aspect of the invention is a compound of formula (I) or a salt thereof as hereinbefore defined for use in a method of treatment of the human or animal body by surgery or therapy, or of diagnosis practiced on the human or animal body. In particular the present invention provides a compound of formula (I) or a salt thereof as hereinbefore defined for use in the treatment of cancer.

The invention also provides the use of a compound of formula (I) or a salt thereof as hereinbefore defined in the manufacture of a medicament for use in the treatment of cancer.

Compounds of formula (I) are prepared by a two stage process. In the first stage a bis(chloroalkanamido) anthraquinone compound is prepared, which is subjected to further treatment in the second stage of the process. This bis(chloroalkanamido)anthraquinone intermediate is a compound of general formula (II):



(II)

in which n is 1, 2 or 3.

The first stage of the process to prepare compounds of formula (I) comprises treating 2,6-diaminoanthraquinone with a chloroalkanoyl chloride of formula $Cl(CH_2)_nCOCl$ in which n is 1, 2 or 3. This leads to the formation, in crude form, of a compound of formula (II) as defined above. The reaction is carried out either in neat chloroalkanoyl chloride or in the presence of an organic solvent. Generally when n is 1 or 2 the reaction is carried out in neat chloroacetyl chloride or neat 3-chloropropanoyl chloride respectiv ly. When n is 3 the 2,6-diamino-

chloride respectively. When n is 3 the 2,6-diamino-anthraquinone is typically first suspended in an organic solvent, such as benzene, to which the chloroalkanoyl chloride is then added. The process is carried out with heating, typically under reflux conditions. Heating is generally continued for up to 6 hours. Upon completion of the reaction and removal of excess acylating agent, the crude product of formula (II) may be used directly in the second stage of the process without prior purification.

The second stage of the process to prepare compounds of formula (I) involves aminolysis of the bis(chloro-alkanamido)anthraquinone intermediate produced in the first stage. This comprises treating a compound of formula (II) as defined above, with the exception of a compound where n = 3, with, in the presence of an organic solvent, an amine of formula HNR^1R^2 in which R^1 and R^2 are the same or different and are each as defined above in connection with formula (I).

The crude compound (II) is generally first suspended in a solvent, for example an alcohol such as ethanol. The suspension is typically stirred and heated to gentle reflux. The amine of formula HNR^1R^2 is then added, usually dropwise and usually over a period of 5 to 40 minutes. The reaction mixture is then refluxed for sufficient time for the reaction to reach completion, as evidenced by TLC. This may take anything from 3 to 30 hrs, typically from 5 to 14 hrs.

When the reaction is complete the compound of formula (I) is isolated from the crude product mixture and purified by suitable standard methods.

The purified base compound may be converted to a salt. The preferred salt is either the acetate, in which case each of the basic amine functions $-NR^1R^2$ in formula (I) is converted to the group $-NR^1R^2.HOAC$; or it is the quaternary methylammonium salt, in which case the amine

functions are converted to the group $-N^+(CH_3)R^1R^2I^-$. Other suitable salts include the succinate and the maleate.

To prepare the acetate salt, the base compound (I) is typically suspended in glacial acetic acid, treated with activated charcoal and heated. The resulting solution is filtered and trituration of the filtrate yields the acetate salt as a brightly coloured precipitate.

To prepare the methylammonium salt, the base compound (I) is typically suspended in acetone, treated with CH_3I and stirred at room temperature for a day.

The products of the reaction are then filtered, washed with diethyl ether and dried.

The following Table 1 lists the compounds and salts synthesised in accordance with the invention.

TABLE 1

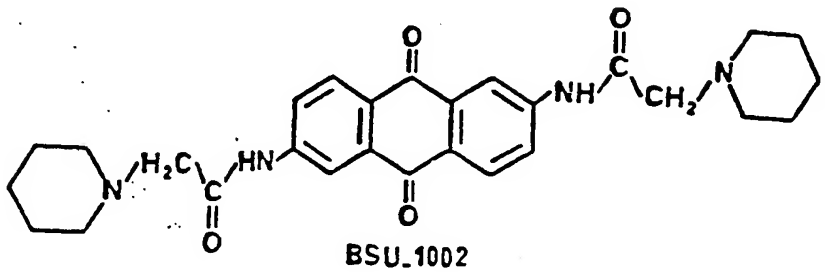
BASE COMPOUND OF FORMULA (I)	n	BASIC AMINE FUNCTION $-NR^1R^2$	ACETATE SALT $-NR^1R^2.HOAC$	METHYLAMMONIUM SALT $-N^+(CH_3)R^1R^2I^-$
BSU-1002	1	-PIPERIDINE	BSU-1032[a]	BSU-1003
BSU-1004	1	-MORPHOLINE	BSU-1022[a]	BSU-1005
BSU-1006	1	-DIETHYLAMINE	BSU-1024	BSU-1007
BSU-1008	1	-4-METHYL-PIPERAZINE	BSU-1025	BSU-1034
BSU-1015	2	-PIPERIDINE	BSU-1021	BSU-1026
BSU-1016	2	-MORPHOLINE	BSU-1028[a]	BSU-1027
BSU-1017	2	-DIETHYLAMINE	BSU-1030	BSU-1029
BSU-1018	2	-4-METHYL-PIPERAZINE	BSU-1031	NS
BSU-1035	2	-2-(2-HYDROXY-ETHYL)PIPERIDINE	BSU-1037	BSU-1036
BSU-1038	2	-4-(2-HYDROXY-ETHYL)PIPERIDINE	BSU-1042	NS
BSU-1039	2	-4-(2-HYDROXY-ETHYL)PIPERAZINE	BSU-1043	NS
BSU-1040	2	-2-HYDROXYMETHYL-PIPERIDINE	BSU-1044	NS
BSU-1041[b]	2	-N,N-DIETHANOL-AMINE	NS	NS

a - sparingly water-soluble salts

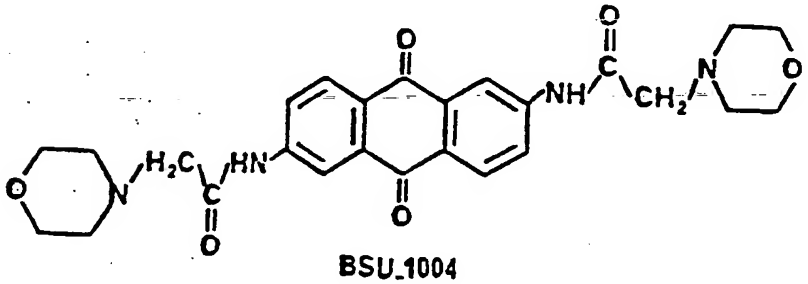
b - water-soluble base, hence no salt synthesised

NS - not synthesised

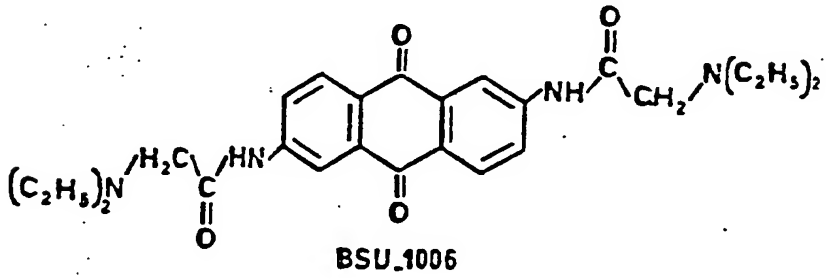
The structural formulae of the base compounds (I) synthesised are as follows:



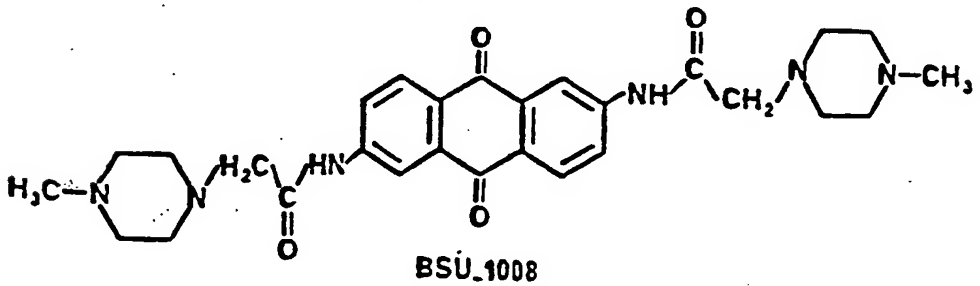
BSU_1002



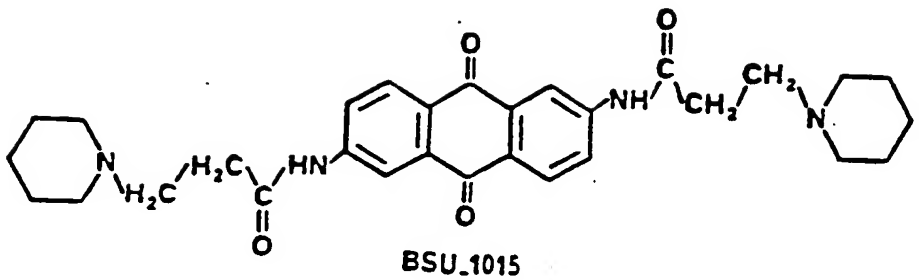
BSU_1004



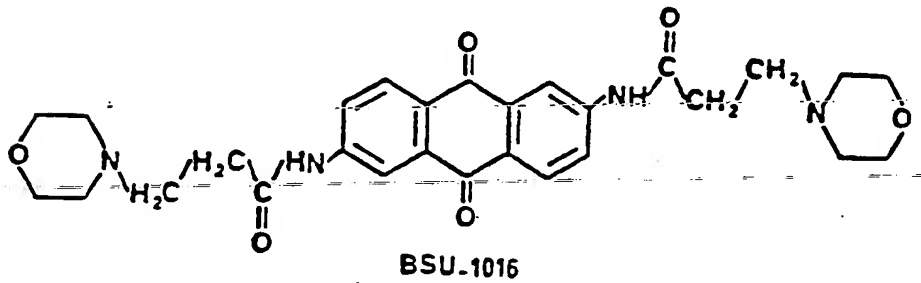
BSU_1006



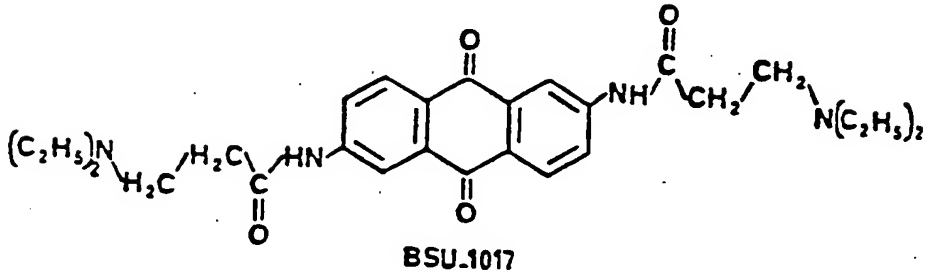
BSU_1008



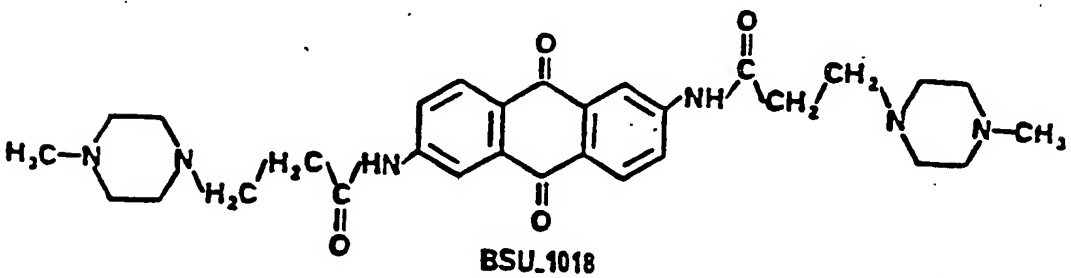
BSU_1015



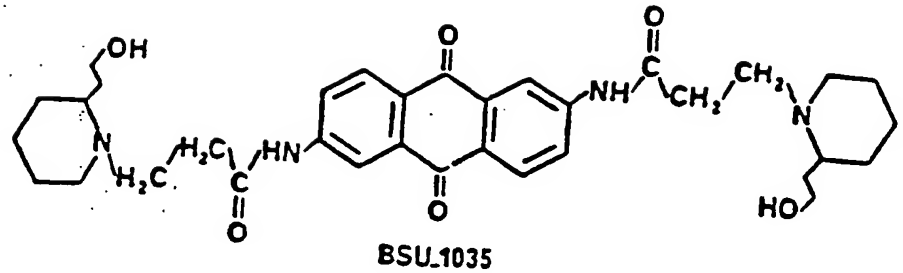
BSU_1016



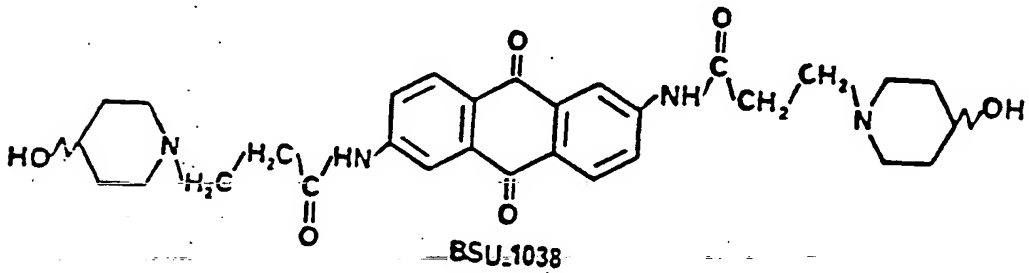
BSU_1017



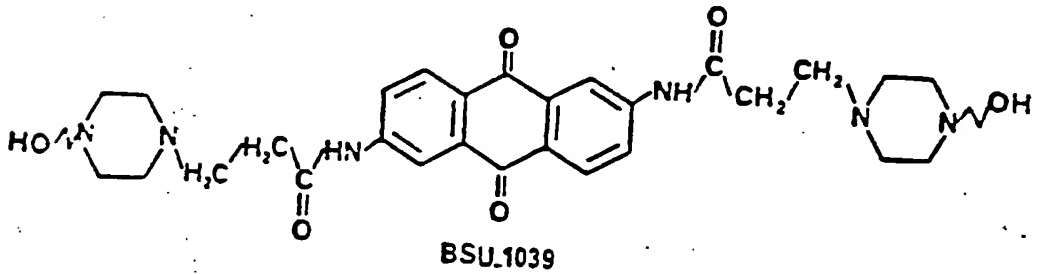
BSU_1018



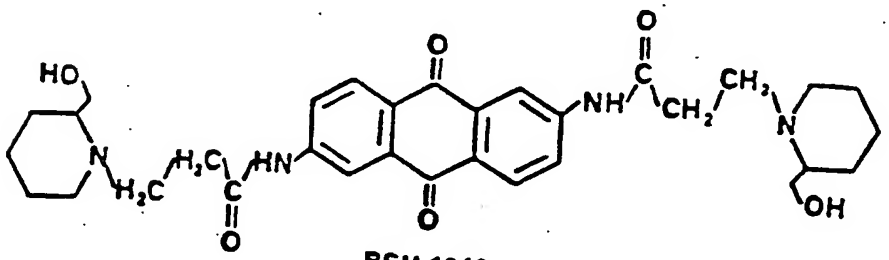
BSU_1035



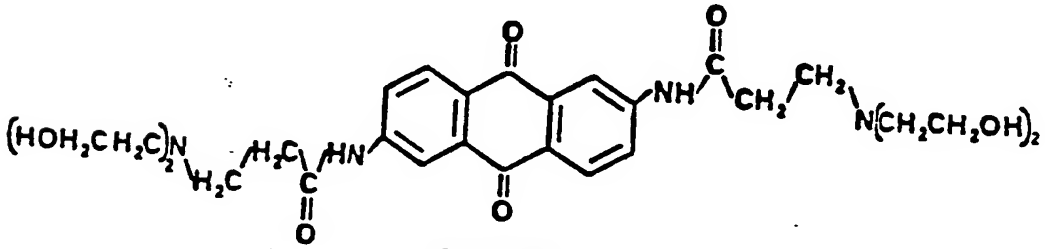
BSU_1038



BSU_1039



BSU_1040



BSU_1041

The compounds of formula (I) and their salts have pharmaceutical utility in that they possess properties making them suitable as anti-cancer agents. They interact with DNA; they are cytotoxic (with the exception of the compound BSU-1022); and they are effectively non-mutagenic.

The use of biophysical techniques and molecular modelling has shown that interaction with DNA occurs by a mode of binding which is similar to that of certain known anti-cancer agents which may exert their effect by intercalation in the double helix of DNA. The DNA binding properties of some compounds and salts of formula (I) are shown in Table 2 below:

TABLE 2

COMPOUND NO.	K^a ($\times 10^6$, $dm^3\ mol^{-1}$)	n^b	ω^c
BSU-1025	20.7	2.830	0.077
BSU-1021	78.4	3.648	0.229
BSU-1037	0.47	4.100	0.399
BSU-1030	1.39	4.376	0.319
BSU-1041	3.88	4.100	0.228

a - affinity contants calculated using the extended McGhee-von Hippel model

b - the number of base pairs occluded by each bound drug molecule, that is, the binding site size

c - the co-operativity factor

It is to be noted that the poor aqueous solubility of many of the salts of compounds of formula (I), particularly these with n = 1, limits the binding data which can be determined.

The compounds stabilize various DNA's towards thermal denaturation, the increase in Tm for certain n = 2 compounds being comparable to that of mitoxantrone (a known intercalator). Although an increase in Tm is typical of intercalators, it is also seen with groove binders. DNA modelling studies carried out to establish possible intercalative binding modes have shown, however, that the compounds can only intercalate into DNA with one side-chain of the molecule residing in each DNA groove. The n = 2 compounds are shown to be better intercalators than the n = 1 derivatives since the longer side chains afford greater interaction with the sugar-phosphate backbone of the DNA molecule. Intercalative binding is also shown to be enhanced by the existence of hydrogen bonding which can occur when the side chains of the compounds of formula (I) bear OH substituents.

The compounds of formula (I) and the salts thereof are also shown to be capable of unwinding covalently-closed supercoiled plasmid PM2 DNA. Only the diethanolamine-substituted compound BSU-1041 does not cause positive supercoiling of the plasmid DNA, even at high concentrations. The unwinding angles observed are comparable to these determined for known intercalators and lend further support to intercalation being the major mode of binding.

The compounds of formula (I) and their salts are all cytotoxic towards L1210 leukaemia and WS tumour cells and Chinese hamster cells as determined in vitro at low concentrations. The n = 2 compounds are more cytotoxic than the n = 1 analogues and show, for example, a 10-fold differential toxicity towards L1210 cells in vitro compared to Walker or V79 cells. This is particularly marked with the compounds having non-hydroxy substituted side-chains. The known intercalator mitoxantrone also exhibits such differential toxicity.

The in vitro studies have shown that biological activity is dependent upon the protonation status of the terminal amine residues in the side-chains of compounds of formula (I). The more basic compounds are the more active. In vitro toxicity is also influenced, in common with the DNA binding, by the length of the compounds' side-chains, with n = 2 compounds being the more active. There therefore appears to be a qualitative correlation between the in vitro activity and the DNA binding, as evidenced by ΔTm data, of the compounds of formula (I).

Studies in vivo show two of the n = 2 compounds, BSU-1043 and BSU-1042, to have marginal activity against the L1210 tumour model system. The n = 1 compound BSU-1008 (evaluated as the acetate salt BSU-1025) is also slightly active in vivo. However, the fact that in vivo results obtained hitherto do not correlate well with the in vitro results may be due to several factors such as pharmacokinetics and/or limited drug penetration. Plasma stability studies have shown the compounds to be stable for upwards of 2 hrs during incubation at 37°C and so it is unlikely that the compounds degrade in vivo.

The third valuable property of the compounds of formula (I) is that they are non-mutagenic. This is shown using the "Ames test" and persists at levels at which mitoxantrone is mutagenic. Mutagenicity is frequently linked with anti-cancer activity and so these results indicate the clinical use of compounds of formula (I) and their salts as anti-cancer agents.

Accordingly, the present invention further provides a method of treating a host suffering from cancer which method comprises administering thereto a pharmaceutically effective amount of a compound of formula (I).

The compounds of the invention can be administered in a variety of dosage form, e.g. orally, in the form of tablets, capsules, sugar or film coated tablets, liquid

soluti ns or suspensions; rectally, in the form of suppositories, parenterally, e.g. intramuscularly, or by intravenous injection or infusion. The dosage depends on the age, weight, condition of the patient, type of tumour to be treated, and administration route. A suitable dosage for oral administration to adult humans is from 0.1 to 100 mg per kg body weight per day, preferably from 1 to 10 mg per kg.

The invention includes pharmaceutical compositions comprising a pharmaceutically acceptable carrier or diluent and, as active principle, a compound of formula (I). The pharmaceutical compositions containing the compounds of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For example, the solid oral forms may contain, together with the active compound, diluents, e.g., lactose, dextrose, saccharose, cellulose, corn starch or potato starch: lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates, or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Said pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film coating processes.

The liquid dispersions for oral administration may be e.g. syrups, emulsions and suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

The susp nsions and the emulsi ns may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

The solutions for intravenous injections or infusions may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

The suppositories may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. cocoa-butter, polyethylene glycol, a polyoxyethylene sorbitan fatty acid ester surfactant or lecithin.

The following Examples further illustrate the invention. In Examples 1 to 18, the compounds were characterised using the following methods. IR spectroscopy was performed on a Perkin Elmer IR model 1310 spectrophotometer using Nujol mulls or pressed KCl discs; proton magnetic resonance (^{1}H-NMR) spectra were recorded using a BRUKER AC250 250MHz FT-NMR instrument operating at 293±1K; UV-visible spectra were recorded using a Varian Cary 219 spectrophometer, and mass spectra were recorded using a VG7070H mass spectrometer fitted with EI(electron-impact) ionisation source (70eV, 390-420K source temperature). The fast atom bombardment (FAB) ionisation method was used for the determination of the mass spectra of certain compounds of poor solubility or volatility.

EXAMPLE 1

2,6-Bis(2-chloroacetamido)anthracene-9,10-dione
(BSU-1001)

2,6-Diaminoanthracene-9,10-dione (30.4 g, 0.128 mol) was refluxed in neat chloroacetyl chloride (500 g, 4.43 mol) for a period of 3 hrs, after which time the reaction was shown to have reached completion by TLC. The reaction mixture was observed to change colour from the brick-red of the diamine to mustard yellow during the course of the reaction. The reaction mixture was cooled in an external ice-water bath, filtered and the filter cake was washed thoroughly with toluene to afford an essentially quantitative yield of the title compound. Yield: 50.07 g (~100%th.). R_f(EtOH)=0.84. A small amount of this material was recrystallised from ethanol for characterisation, m.p. 310-311°C. The crude product was used in the synthesis of the final amide-substituted anthraquinones with n = 1 without further purification.

Anal. ($C_{18}H_{12}N_2O_4Cl_2$):
calcd. C 55.26 H 3.09 N 7.16 Cl 18. 13%,
found C 55.35 H 3.33 N 7.02 Cl 17.96%.

IR (Nujol) 3370(NH), 3335, 3300, 1722(amide C=O), 1685(quinone C=O) and 1590 cm^{-1}; ^{1}H-NMR (Me_2SO-d_6) δ 4.35 (s, 4H, CH_2Cl), 8.07 (dd, J = 2.1 and 8.5 Hz, 2H, H-3,7), 8.20 (d, J = 8.5 Hz, 2H, H-4,8), 8.44 (d, J = 2.1 Hz, 2H, H-1,5), 10.90 (s, 2H, D_2O removes, NH).

EXAMPLE 2

2,6-Bis(3-chloropropionamido)anthracene-9,10-dione (BSU-1013)

2,6-Diaminoanthracene-9,10-dione (30.0 g, 0.126 mol) was refluxed in neat 3-chloropropanoyl chloride (500 g, 3.94 mol) for a period of 5 hrs, after which time the reaction was judged to have reached completion by TLC. The reaction mixture was observed to change colour from the brick red of the diamine to mustard yellow within 30 mins of reflux. The reaction mixture was cooled in an external ice-water bath and then filtered under vacuum. The solid product was washed with anhydrous diethyl ether (4 x 100 ml), followed by resuspension in anhydrous diethyl ether (2 x 200 ml), and final washing with 1,4-dioxane (2 x 400 ml). The solid material was dried under vacuum, washed once again with anhydrous diethyl ether and finally left to dry at room temperature. Yield: 51.51 g (95% th). R_f(EtOH)= 0.84. A small amount of this crude product was recrystallised from DMF:EtOH (4:1 v/v) for characterisation. The melting point of the recrystallised title compound was >340°C. The crude material was used in the synthesis of the final aminoalkanamido-substituted anthraquinones with n = 2 without further purification.

Anal. ($C_{20}H_{16}N_2O_4Cl_2$):

calcd. C 57.29 H 3.85 N 6.68 Cl 16.91%,

found C 56.91 H 4.23 N 6.76 Cl 16.28%.

NMR (DMSO-d_6, δppm): 2.93(t, J 6.2 Hz, 4H, -CO$\underline{CH_2}$-), 3.92 (t, J 6.2 Hz, 4H, -$\underline{CH_2}$Cl), 8.07(dd, J 1.8 and 8.5 Hz, 2H, arom.H3,7), 8.16(d, J 8.5 Hz, 2H, arom.H4,8), 8.44(d, J 1.8 Hz, 2H, arom.H1,5), 10.77(s, 2H, D_2O removes, -N$\underline{H}$CO-).

IR (kBr, ν cm^{-1}): 3372(amide NH str.), 3245+3170(C=O str. overtones), 1703(amide C=O str.), 1657(quinone C=O str.), 1582(arom. C=C str.), etc.

EXAMPLE 3

2,6-Bis(4-chlorobutyramido)anthracene-9,10-dione
(BSU-1009)

2,6-Diaminoanthracene-9,10-dione (30.0 g, 0.126 mol) was suspended in 1000 ml of benzene. 4-Chlorobutyryl chloride (500 ml, 4.46 mol) and a catalytic amount of pyridine (~1 g) were added and the suspension was heated for 4 hrs at 70°C. The solvent was removed to give an essentially quantitative yield of the bis-amide. Yield: 55.8 g (≙99%th). R_f(EtOH)=0.90. A small amount of the crude product was recrystallised from DMF:EtOH (4:1 v/v) for characterisation, m.p. >340°C. The crude product was used in the attempted synthesis of the final amino-alkanamido-anthraquinones with n=3 without further purification. Anal. ($C_{22}H_{20}N_2O_4Cl_2$):

calcd. C 59.07 H 4.48 N 6.27 Cl 15.86%,
found C 58.95 H 4.70 N 6.08 Cl 15.36%.

NMR (DMSO-d_6, δ ppm): 2.07(broad quintet, mean J 6.8 Hz, 4H, $-CH_2\underline{CH_2}CH_2-$), 2.59(t, J 7.2 Hz, 4H, $-NHCO\underline{CH_2}CH_2-$), 3.73(t, J 6.5 Hz, 4H, $-CH_2\underline{CH_2}Cl$), 8.06(dd, J 1.8 and 8.5 Hz, 2H, arom.H3,7), 8.15(d, J 8.5 Hz, 2H, arom.H4,8), 8.44(d, J 1.8 Hz, 2H, arom.H1,5), 10.66(s, 2H, D_2O removes, $-\underline{NH}CO-$).

IR (Nujol; ν cm^{-1}): 3350(amide NH str.), 3300, 3240, 3175, 3110, 1706(amide C=O str.), 1660(quinone C=O str.), 1575(arom. C=C str.), etc.

EXAMPLE 4

2,6-Bis(2-(1-piperidino)acetamido)anthracene-9,10-dione (BSU-1002)

2,6-Bis(2-chloroacetamido)anthracene-9,10-dione (BSU-1001, 10.0 g, 25.6 mmol) was suspended in ethanol (300 ml) with vigorous stirring and heated to gentle reflux. Piperidine (30 ml, 0.30 mol) was added dropwise to the suspension over a period of 15 mins. The reaction mixture was refluxed for a period of 5 hrs, after which time the reaction was shown to have reached completion by TLC. The reaction mixture was observed to change appearance from an initial yellow suspension to a dark brown mixture within 30 mins of reflux. The reaction mixture was cooled in an ice-water bath and then filtered. The solid product was washed with anhydrous diethyl ether (4 x 100 ml), filtered and dried at 20°C. The solid material was digested in chloroform (150 ml) to give a yellowish-brown solution, treated with activated charcoal (~0.5 g) and filtered. Rotary evaporation under reduced pressure afforded a yellowish-green powder. The solid product was recrystallised from chloroform-ethanol (3:2 v/v) to give the title compound as yellowish-green crystals (7.73 g, 62%th), m.p. 235-236°C.

R_f(EtOH)=0.52.

Anal. ($C_{28}H_{32}N_4O_4$):

calcd. C 68.83 H 6.60 N 11.47%,

found C 68.62 H 6.53 N 11.25%.

NMR (DMSO-d_6, δ ppm): 1.30-1.42(m, 4H, $-(CH_2)_2\underline{CH_2}(CH_2)_2-$), 1.55-1.58(m, 8H, $-NCH_2\underline{CH_2}CH_2-$), 2.46-2.50(m, 8H, $-N\underline{CH_2}(CH_2)_2-$), 3.16(s, 4H, $CO\underline{CH_2}N<$), 8.14(dd, J 2.1 and 8.0 Hz, 2H, arom.H3,7), 8.17(d, J 8.0 Hz, 2H, arom.H4,8), 8.54 (d, J 2.1 Hz, 2H, arom.H1,5), 10.39(s, 2H, D_2O removes, $-N\underline{H}CO-$).

IR (Nujol, cm^{-1}): 3295(amide NH str.), 3200, 3100, 3055, 1700(amide C=O str.), 1673(quinone C=O str.), 1600, 1580(aromatic C=C str.), etc.

MS, m/z (rel. intensity): 488($[M]^{\cdot+}$, 4%), 405 ($[M-C_5H_{10}N]^{\cdot+}$, 6%), 98($[C_7H_{12}N]^{\cdot+}$,100%) 84($[C_5H_{10}N]^{\cdot+}$,35%).

Diacetate salt (BSU-1032): mp 210-212°C.

Methiodide salt (BSU-1003): mp 245-247°C dec.

EXAMPLE 5

2,6-Bis(2-(4-morpholino)acetamido)anthracene-9,10-dione (BSU-1004)

2,6-Bis(2-chloroacetamido)anthracene-9,10-dione (BSU-1001, 10.5 g, 26.8 mmol) was suspended in ethanol (300 ml) and the stirred mixture was heated to gentle reflux. Morpholine (25 ml, 0.29 mol) was added dropwise, over a period of 15 mins, to the refluxing solution. The mixture was refluxed for a period of 7 hrs, at which point the reaction was shown to have reached completion by TLC. During the reaction period, the colour of the reaction mixture was observed to change from mustard yellow to greenish yellow. The solvent and volatile organics were removed under reduced pressure. The yellow solid product was digested in chloroform (100 ml), treated with activated charcoal and the solution filtered. The clear chloroform filtrate was subsequently passed through a bed of silica gel (10 cm x 3 cm diameter) to give a bright yellow solution. The eluate was evaporated under reduced pressure to give a bright yellow powder which was thoroughly washed with anhydrous diethyl ether (3 x 400 ml).

Recrystallisation from ethanol-chloroform (1:1 v/v) gave the title compound as yell w crystals (7.99 g, 64% th), m.p. 263-264°C.

R_f(EtOH)= 0.68.

Anal.($C_{26}H_{28}N_4O_6$):

calcd. C 63.40 H 5.73 N 11.38%,

found C 63.13 H 5.74 N 11.18%.

NMR (DMSO-d_6, δ ppm): 2.53(broad s, 8H, -N($\underline{CH_2}CH_2)_2O$), 3.23(s, 4H, -CO$\underline{CH_2}$N<), 3.46(broad s, 8H, -N($CH_2\underline{CH_2})_2O$), 8.16(s, 4H, arom.H3,4,7,8), 8.52(s, 2H, arom.H1,5), 10.45 (s, D_2O removes, 2H, 2H, -N$\underline{H}$CO-).

IR (Nujol, ν cm^{-1}): 3285(amide NH str.), 3115, 3080, 1687 (amide C=O str.), 1669(quinone C=O str.), 1592(aromatic C=C str.), etc.

MS, m/z (rel.intensity): 492($[M]N\cdot^+$,2%), 406($[M-C_4H_8NO]\cdot^+$, 1%), 368(5%), 236(3%), 100($[C_5H_{10}NO]\cdot^+$,100%), 86($[C_4H_8NO]\cdot^+$, 14%), 83(20%).

Diacetate salt (BSU-1022): 262-263°C.

Methiodide salt (BSU-1005): 244-246°C dec.

EXAMPLE 6

2,6-Bis(2-diethylaminoacetamido)anthracene-9,10-dione (BSU-1006)

2,6-Bis(2-chloroacetamido)anthracene-9,10-dione (BSU-1001, 10.5 g, 26.8 mmol) was suspended in ethanol (300 ml) with stirring, and heated to reflux temperature. Diethylamine (25 ml, 0.24 mol) was added dropwise during 15 mins. The reaction mixture was refluxed for 5 hrs, after which time the reaction mixture was cooled using an external ice-water bath and the solid precipitate formed was collected by filtration. The product was thoroughly washed with anhydrous diethyl ether (4 x 100 ml) and dried to give a yellowish-green, amorphous powder.

Recrystallisation from ethanol afforded the title compound as flat green crystals (7.38 g, 62.0%th.), m.p. 191-192°C. R_f(EtOH)= 0.47.

Anal. ($C_{26}H_{32}N_4O_4$):

calcd. C 67.22 H 6.94 N 12.06%,

found C 67.81 H 6.81 N 11.88%.

NMR (DMSO-d_6, δ ppm): 1.03(t, J 7.1 Hz, 12H, $-N(CH_2\underline{CH_3})_2$), 2.63(q, J 7.1 Hz, 8H, $-N(\underline{CH_2}CH_3)_2$), 3.26(s, 4H, $-CO\underline{CH_2}N<$), 8.18(s, 4H, arom.H3,4,7,8), 8.56(s, 2H, arom.H1,5), 10.35 (s, D_2O removes, 2H, $-N\underline{H}CO-$).

IR (Nujol, ν cm^{-1}): 3255(amide NH str.), 3090 1680(amide C=O str.), 1662(quinone C=O str.), 1594(arom. C=C str.), etc.

MS, m/z (rel.intensity): 464($[M]^{\cdot+}$,2%), 449(2%), 436 ($[M-C_2H_4]^{\cdot+}$,1%), 393(2%), 378($[M-C_5H_{12}N]^{\cdot+}$,1%), 86 ($[C_5H_{12}N]^{\cdot+}$,100%), 28($[C_2H_4]^{\cdot+}$,100%).

Diacetate salt (BSU-1024): mp 188-189°C. Anal. ($C_{30}H_{40}N_4O_6$) C,H,N.

Methiodide salt (BSU-1007): mp 240-241.5°C dec.

EXAMPLE 7

2,6-Bis(2-(4-methyl-1-piperazino)acetamido)anthracene-9,10-dione (BSU-1008)

2,6-Bis(2-chloroacetamido)anthracene-9,10-dione (BSU-1001), 10.5 g 26.8 mmol) was suspended in ethanol (300 ml) with stirring and heated to reflux temperature. 4-Methylpiperazine (25 ml, 0.23 mol) was added dropwise during 15 mins. The reaction mixture was refluxed for a period of 5 hrs, after which time the reaction was judged to have reached completion by TLC. The reaction mixture was cooled in an ice-water bath and the solid material was

collected by filtration. The yellow solid was washed thoroughly with anhydrous diethyl ether (4 x 100 ml), digested in chloroform (100 ml) and treated with activated charcoal. After filtration, the solvent was removed under reduced pressure to give a yellow amorphous material. The solid was redigested in chloroform (25 ml), filtered and treated with anhydrous diethyl ether to precipitate the title compound as a brownish-yellow crystalline solid (7.35 g, 55%th.), m.p. 213.5-214°C. R_f(EtOH)=0.34.

Anal. ($C_{28}H_{34}N_6O_4$):

calcd. C 64.84 H 6.61 N 16.21%,

found C 64.76 H 6.56 N 15.90%.

NMR (DMSO-d_6, δ ppm): 2.17(s, 6H, >N-$\underline{CH}_3$), 2.38(broad m, 8H, -$COCH_2N\underline{CH}_2CH_2N$-), 2.50(broad m, 8H, >$NCH_2\underline{CH}_2N$-$CH_3$), 3.19(s, 4H, $CO\underline{CH}_2N$<), 8.11(dd, J 2.0 and 8.6 Hz, 2H, arom.H3,7), 8.17(d, J 8.6 Hz, 2H, arom.H4,8), 8.50(d, J 2.0 Hz, 2H, arom.H1,5), 10.39(s, D_2O removes, 2H, -$N\underline{H}CO$-).

IR (Nujol, ν cm^{-1}): 3345(amide NH str.), 3260, 3180, 1718 (amide C=O str.), 1660(quinone C=O str.), 1578(arom. C=C str.), etc.

MS, m/z (rel.intensity): 518($[M]^{\cdot+}$,49%), 405 ($[M-C_6H_{13}N_2]^{\cdot+}$,6%), 368(20%), 236(6%), 113 ($[C_6H_{13}N_2]^{\cdot+}$,100%), 98($[C_5H_{13}N_2-1]^{\cdot+}$,28%).

Diacetate salt (BSU-1025): mp 215-217.5°C. Anal. ($C_{36}H_{50}N_6O_{12}$) C,H,N.

Methiodide salt (BSU-1034): mp 268.5-270°C dec.

EXAMPLE 8

2,6-Bis(3-(1-piperidino)propionamido)anthracene-9,10-dione (BSU-1015)

2,6-Bis(3-chloropropionamido)anthracene-9,10-dione (BSU-1013, 10.0 g, 23.9 mmol) was suspended in ethanol (300ml) and the stirred mixture was heated to gentle reflux. Piperidine (25 ml, 0.25 mol) was added dropwise during 20 mins. Reflux was continued for 5 hrs, after which time the reaction was judged (TLC) to have reached completion. The reaction mixture was observed to change colour from mustard yellow to dark brown during the course of the reaction. The reaction mixture was cooled in an external ice-water bath and the solid product was collected by filtration. The solid product was thoroughly washed with anhydrous diethyl ether (4 x 100 ml) and dried at 20°C to give a brown amorphous powder. The title compound was recrystallised from dimethylformamide-ethanol (10:1 v/v) with activated charcoal treatment to give a semi-crystalline brown product, m.p. 270-271°C. Yield: 11.95 g (95% th).).

R_f(EtOH)=0.08.

Anal. ($C_{30}H_{36}N_4O_4$):

calcd. C 69.74 H 7.02 N 10.85%,

found C 69.75 H 7.01 N 10.88%.

NMR (DMSO-d_6, δ ppm): 1.39(broad s, 4H, $-(CH_2)_2\underline{CH_2}(CH_2)_2-$), 1.49(broad s, 8H, $-NCH_2\underline{CH_2}CH_2-$), 2.38(broad s, 8H, $-N(\underline{CH_2}CH_2)_2CH_2-$), 2.45-2.70(m, 8H, $-CO\underline{CH_2}CH_2N<$), 8.05(dd, J 2.0 and 8.8 Hz, 2H, arom.H3,7), 8.17(d, J 8.8 Hz, 2H, arom.H4,8), 8.42(d, J 2.0 Hz, 2H, arom.H1,5), 10.84(s, D_2O removes, 2H, $-N\underline{H}CO-$).

IR (Nujol, ν cm^{-1}): 3325(amide NH str.), 3290, 3190, 3115,

1700(amide C=O str.), 1672(quinone C=O str.), 1618, 1590 (aromatic C=C str.) etc.

MS, m/z (rel.intensity): 516([M]·+,4%), 431([M-$C_5H_{10}N$-1]·+, 10%), 346(63%), 318(10%), 292(37%), 238(30%), 112 ([$C_7H_{14}N$]·+,4%), 98([$C_6H_{12}N$]·+,40%), 84([$C_5H_{10}N$]·+,56%), 83(90%).

Diacetate salt (BSU-1021): mp 244-246°C dec. Anal. ($C_{34}H_{44}N_4O_8$) C,H,N.

Methiodide salt (BSU-1026): mp 262-264.5°C dec.

EXAMPLE 9

2,6-Bis(3-(4-morpholino)propionamido)anthracene-9,10-dione (BSU-1016)

2,6-Bis(3-chloropropionamido)anthracene-9,10-dione (BSU-1013, 10.0 g, 23.9 mmol) was suspended in ethanol (300 ml) with stirring and heated to gentle reflux. Morpholine (40 ml, 0.46 mol) was added dropwise during 30 mins to the refluxing solution. Reflux was continued for 17 hrs, after which time the reaction was judged to have reached completion by TLC. The colour of the reaction mixture was observed to change from mustard yellow to dark brown during the reaction period. The reaction mixture was cooled in an external ice-water bath and the solid product was collected by filtration. The crude material was washed thoroughly with anhydrous diethyl ether (4 x 100 ml) and dried at room temperature. Recrystallisation from dimethylformamide-ethanol (4:1 v/v), with activated charcoal treatment, afforded the title morpholino-compound as a yellowish-brown amorphous powder (10.54 g, 83%th.), m.p. 270.5-271.5°C. R_f(EtOH)=0.15.

Anal. ($C_{28}H_{32}N_4O_4$):
calcd. C 64.60 H 6.20 N 10.76%,
found C 64.64 H 6.00 N 10.59%.
NMR (DMSO-d_6, δ ppm): 2.42(t, J 4.5 Hz, 8H, >N($\underline{CH_2}CH_2)_2O$), 2.55-2.68(AB quartet, 4H, -CO$\underline{CH_2}\underline{CH_2}$N<), 3.57(t, J 4.5 Hz, 8H, -N($\underline{CH_2}CH_2)_2O$), 8.07(dd, J 2.0 and 8.6 Hz, 2H, arom.H3,7), 8.17(d, J 8.6 Hz, 2H, arom.H4.8), 8.44(d, J 2.0 Hz, 2H, arom.H1,5), 10.70(s, D_2O removes, 2H, -N$\underline{H}$CO-).
IR (Nujol, ν cm^{-1}): 3325(amide NH str.), 3290, 3195, 3120, 1698(amide C=O str.), 1670(quinone C=O str.), 1613, 1589 (arom. C=C str.), etc.
MS, m/z (rel.intensity): 520([M]$\cdot^+$,2%), 433([M+1-C_4H_8NO]$\cdot^+$,3%), 346(52%), 292(29%), 238(30%), 100([$C_5H_{10}NO$]$\cdot^+$,37%), 86 ([C_4H_8NO]$\cdot^+$,100%).
Diacetate salt (BSU-1028): mp 255-257°C.
Methiodide salt (BSU-1027): mp 232-233°C.

EXAMPLE 10

2,6-Bis(3-diethylaminopropionamido)anthracene-9,10-dione (BSU-1017)

2,6-Bis(3-chloropropanamido)anthracene-9,10-dione (BSU-1013, 10.0 g, 23.9 mmol) was suspended in ethanol (300 ml) and the stirred mixture was heated to reflux. Diethylamine (25 ml, 0.24 mol) was added dropwise during 15 mins to the refluxing solution. The reaction mixture was refluxed for a period of 5 hrs, after which time the reaction was judged to have reached completion by TLC. The colour of the reaction mixture was observed to change from mustard yellow to bright yellow during the course of the reflux. The reaction mixture was cooled using an external ice-water bath and the solid material was collected by

filtration. The crude solid product was washed thoroughly with anhydrous diethyl ether (4 x 100 ml) and dried at room temperature. Recrystallisation from dimethylformamide-ethanol (4:1 v/v), with activated charcoal (~0.5 g) treatment, afforded the title propionamido derivative as bright yellow crystals (10.41 g, 88%th.), m.p. 223-225°C. R_f(EtOH)=0.55.

Anal. ($C_{28}H_{36}N_4O_4$):

calcd. C 68.27 H 7.37 N 11.27%,

found C 67.72 H 7.24 N 11.46%.

NMR (DMSO-d_6, δ ppm): 0.98(t, J 7.1 Hz, 12H, $>N(CH_2\underline{C}H_3)_2$), 2.50(q, J 7.1 Hz and t, J 6.9 Hz, 12H, $-N(\underline{C}H_2CH_3)_2$ and $-CO\underline{C}H_2CH_2N<$), 2.77(t, J 6.9 Hz, 4H, $-COCH_2\underline{C}H_2N<$), 8.05(dd, J 2.0 and 8.5 Hz, 2H, arom.H3,7), 8.16(d, J 8.5 Hz, 2H, arom.H4,8), 8.42(d, J 2.0 Hz, 2H, arom.H1,5), 10.75(s, D_2O removes, 2H, $-N\underline{H}CO-$).

IR (KCl, ν cm^{-1}): 3315(amide NH str.), 3280, 3185, 3110, 3055, 1698(amide C=O str.), 1669(quinone C=O str.), 1611, 1586(arom. C=C str.), etc.

MS, m/z (rel.intensity): 493($[M+1]\cdot^+$,100%), 492($[M]\cdot^+$,29%), 461(29%), 369(80%), 364($[M-C_7H_{14}NO]\cdot^+$,14%), 277(100%), 186(100%), 185(100%), 82(26%).

Diacetate salt (BSU-1030): mp 201-203°C dec.

Methiodide salt (BSU-1029): mp 242.5-243.5°C.

EXAMPLE 11

2,6-Bis(3-(4-methyl-1-piperazino)propionamido)-anthracene-9,10-dione

(BSU-1018)

2,6-Bis(3-chloropropionamido)anthracene-9,10-dione (BSU-1013, 8.5 g, 20.3 mmol) was suspended in ethanol (300 ml) with stirring and heated to reflux temperature. 4-Methylpiperazine (25 ml, 0.23 mol) was added dropwise during 15 mins. Reflux was continued for a total of 9 hrs, after which time the reaction was judged to have attained completion (TLC). The colour of the reaction mixture was observed to change from mustard yellow to greenish-yellow during the reflux period. The reaction mixture was cooled in an external ice-water bath and the crude solid product was washed thoroughly with anhydrous diethyl ether (5 x 200 ml) and dried at room temperature. Recrystallisation from dimethylformamide, with activated charcoal treatment, gave the title compound as a greenish-yellow powder (10.9 g, ~100%th.), m.p. 281-282°C. R_f(EtOH)=0.01.

Anal. ($C_{30}H_{38}N_6O_4.1.5H_2O$):

calcd: C 62.81 H 7.20 N 14.65%,

found C 62.80 H 6.76 N 14.99%.

NMR (DMSO-d_6, δ ppm): 2.14(s, 6H, >N-$\underline{CH_3}$), 2.3-2.7(broad, m, interference from HOD, ~24H, remaining aliphatic H), 8.00(dd, J 2.1 and 8.5 Hz, 2H, arom.H3,7), 8.17(d, J 8.5 Hz, 2H, arom.H4,8), 8.43(d, J 2.1 Hz, 2H, arom.H1,5), 10.76(s, D_2O removes, 2H, -N$\underline{H}$CO-).

IR (KCl, ν cm^{-1}): 3340(amide NH str.), 3300, 3105, 3060, 1699(amide C=O str.), 1662(quinone C=O str.), 1617, 1580 (arom. C=C str.), etc.

MS, m/z (rel.intensity): 547($[M]\cdot^+$,100%), 546($[M]\cdot^+$,31%), 461(3%), 369(100%), 364(100%), 448($[M+1-C_5H_{11}N_2]\cdot^+$,17%),

447([M-$C_5H_{11}N_2$]$^{\cdot+}$,17%), 369(100%), 364(55%).
Tetra-acetate salt (BSU-1031): mp 207-209°C.

EXAMPLE 12

2,6-Bis(3-(2-(2-hydroxyethyl)-1-piperidino)-propionamido)anthracene-9,10-dione (BSU-1035)

2,6-Bis(chloropropionamido)anthracene-9,10-dione (BSU-1013, 4.0 g, 9.54 mmol) was suspended in ethanol (300 ml) with stirring and heated to gentle reflux. 2-(2-Hydroxyethyl)piperidine (10.7 g, 83 mmol) in ethanol (100 ml) was added dropwise during 30 mins. Reflux was continued for 16 hrs, after which time the reaction was judged to have reached completion by TLC. The colour of the reaction mixture was observed to change from mustard yellow to dark brown during the reaction period. The mixture was cooled using an external ice-water bath and the crude product was collected by filtration. The solid was washed thoroughly with anhydrous diethyl ether (4 x 100 ml), water (2 x 100 ml) and chloroform (2 x 100 ml). The product was digested in dimethylformamide (15 ml), treated with activated charcoal (~0.5 g) and filtered (Whatman No. 1 paper) to give a yellowish-brown solution. The addition of acetone resulted in a cloudy precipitate. The mixture was left overnight at 0-5°C to complete precipitation of the title compound as a brown solid (2.98 g, 50%th.), m.p. 211-212°C. R_f(EtOH)=0.08.
Anal. ($C_{34}H_{44}N_4O_6.0.5H_2O$):

calcd.	C 66.54	H 7.39	N 9.13%,
found	C 66.37	H 7.07	N 9.24%.

NMR (DMSO-d_6, δ ppm): 1.28(broad m, 4H, -N(CH_2)$_2$$\underline{CH_2}$-), 1.4-1.9(m, 12H, -$\underline{CH_2}CH_2OH$ and >N$CH_2\underline{CH_2}CH_2\underline{CH_2}$-), 2.25-2.32

(m, 4H, -CO$\underline{CH_2}$-), 2.50-2.54(m, 4H, >N$\underline{CH_2}(CH_2)_2$-), 2.64-2.80 (m, 4H, -COCH$_2\underline{CH_2}$-), 2.89-2.97(m, 2H, >N$\underline{CH}(CH_2)_2$-), 3.40-3.49(m, 4H, -$CH_2\underline{CH_2}$OH), 4.44(broad s, D_2O removes, 2H, -$CH_2\underline{OH}$), 8.07(dd , $\underline{J}$ 2.1 and 8.5 Hz, 2H, arom.H3,7), 8.17 (d, $\underline{J}$ 8.5 Hz, 2H, arom.H4.8), 8.42(d, $\underline{J}$ 2.1 Hz, 2H, arom.H1,5), 10.80(s, D_2O removes, 2H, -N$\underline{H}$CO-).
IR (KCl, ν cm^{-1}): ~3400(broad, OH str.), 3300(broad, amide NH str.), 3180, 3106, 3050, 1695(amide C=O str.), 1668(quinone C=O str.), 1606, 1584(arom. C=C str.), etc.
MS, $\underline{m/z}$ (rel.intensity): 605($[M+1]^{\cdot+}$,100%), 604($[M]^{\cdot+}$,26%), 588($[M+1-OH]^{\cdot+}$,22%), 577($[M+1-C_2H_4]^{\cdot+}$, 11%), 576($[M-C_2H_4]^{\cdot+}$,10%), 560($[M+1-C_2H_5O)^{\cdot+}$,20%), 559($[M-C_2H_5O)^{\cdot+}$,15%), 448($[M-C_9H_{15}NO]^{\cdot+}$,20%), 421($[M+1-C_{10}H_{18}NO_2]^{\cdot+}$,21%), 420($[M-C_{10}H_{18}NO_2]^{\cdot+}$,14%), 406($[M+1-C_{10}H_{19}N_2O_2]^{\cdot+}$,18%), 405($[M-c_{10}H_{19}N_2O_2)^{\cdot+}$,18%).
Diacetate salt (BSU-1037): mp 178-179°C.

EXAMPLE 13

2,6-Bis(3-(4-(2-hydroxyethyl)-1-piperidino)-propionamido)anthracene-9,10-dione (BSU-1038)

2,6-Bis(3-chloropropionamido)anthracene-9,10-dione (BSU-1013, 6.0 g, 14.3 mmol) was suspended in ethanol (300 ml) with stirring and the mixture was heated to gentle reflux. 4-(2-Hydroxyethyl)piperidine (15.0 g, 0.12 mol) in ethanol (100 ml) was added dropwise during 30 mins. The reaction mixture was refluxed for 5 hrs, after which time the reaction was judged (TLC) to have reached completion. The colour of the reaction mixture was observed to change from mustard yellow to dark brown during the reflux period. The mixtur was cooled using an external ice-water bath and the solid material was collect d by filtration. The crude

product was washed th roughly with anhydrous diethyl ether (5 x 100 ml) and dried at 20°C. The product was digested in dimethylformamide (15 ml), treated with activated charcoal and filtered to give a yellowish-brown solution. Trituration with anhydrous diethyl ether gave the title compound as a light brown solid (8.08 g, 93%th.), m.p. 237-237.5°C. R_f(EtOH)=0.03.

Anal. ($C_{34}H_{44}N_4O_6$):

calcd. C 67.53 H 7.33 N 9.26%,

found C 67.11 H 7.38 N 9.37%.

NMR (DMSO-d_6, δ ppm): 1.0-1.2(broad m, 8H, $-NCH_2\underline{CH_2}CH-$), 1.35(t, J 6.3 Hz, 4H, $-\underline{CH_2}CH_2OH$), 1.62+1.66(m, 2H, stereoisomeric $-\underline{CH}CH_2CH_2OH$), 1.92(t, J 11.3 Hz, 8H, $-N\underline{CH_2}\underline{CH_2}\underline{CH}-$), 2.5-2.9(AB quartet, 8H, $-CO\underline{CH_2}\underline{CH_2}N<$), 3.42 (t, J 6.3 Hz, 4H, $-CH_2\underline{CH_2}OH$), 4.35(broad m, D_2O removes, 2H, $-CH_2\underline{OH}$), 8.06(dd, J 2.2 and 8.6 Hz, 2H, arom.H3,7), 8.17(d, J 8.6 Hz, 2H, arom.H4,8), 8.42(d, J 2.2 Hz, 2H, arom.H1,5), 10.81(s, D_2O removes, 2H, $-N\underline{H}CO-$).

IR (KCl, ν cm^{-1}): ν_{3450}(broad, OH str.), 3360(amide NH str.), 3140, 3080, 1720(amide C=O str.), 1682(quinone C=O str.), 1626, 1600(arom. C=C str.), etc.

MS, m/z (rel.intensity): 605($[M+1]^{\cdot +}$>40%), 604($[M]^{\cdot +}$, 21%), 603($[M-1]^{\cdot =}$,7%), 575($[M-1-C_2H_4]^{\cdot +}$,4%), 495(23%), 461($[M-1-C_8H_{16}NO]^{\cdot +}$,10%), 391(12%), 369(15%).

Diacetate salt (BSU-1042): mp 203-205°C dec.

EXAMPLE 14

2,6-Bis(3-(4-(2-hydroxyethyl)-1-piperazino)-propionamido)anthracene-9,10-dione
(BSU-1039)

2,6-Bis(chloropropionamido)anthracene-9,10-dione (BSU-1013, 6.0 g 14.3 mmol) was suspended in ethanol (300 ml) with stirring and heated to gentle reflux. 4-(2-Hydroxyethyl)piperazine (25.0 g, 0.25 mol), in ethanol (100 ml) was added dropwise during 30 mins. Reflux was continued for 14 hrs, after which time the reaction was judged to have reached completion by TLC. The colour of the mixture was observed to change from mustard yellow to bright yellow during the course of reflux. The mixture was cooled in an ice-water bath and the solid product was collected by filtration. The crude material was washed thoroughly with anhydrous diethyl ether (5 x 100 ml) and dried at room temperature. The product was digested in dimethylformamide (15 ml), treated with activated charcoal and filtered to give a yellow solution. Addition of anhydrous diethyl ether resulted in the precipitation of the title compound as a yellow-coloured powder (8.65 g, ~100%th.), m.p. 238-239°C. R_f(EtOH)=0.09.

Anal. ($C_{32}H_{42}N_6O_6.1.0H_2O$):

calcd. C 61.52 H 7.10 N 13.45%,

found C 61.70 H 6.62 N 13.55%.

NMR (DMSO-d_6, δ ppm): 2.35(t, J 6.3 Hz, 4H, -N$\underline{CH_2}CH_2OH$), 2.4-2.5(m, 16H, -N($\underline{CH_2}$)$_2$N-), 2.55-2.65(AB quartet, 8H, -CO$\underline{CH_2}\underline{CH_2}$-), 3.47(t, J 6.3 Hz, 4H, >N$CH_2\underline{CH_2}OH$), 4.40(s, 2H, D_2O removes, -(CH_2)$_2$O$\underline{H}$), 8.05(dd, J 2.0 and 8.4 Hz, 2H, arom.H3,7), 8.21(d, J 8.4 Hz, 2H, arom.H4,8), 8.43(d, J 2.0 Hz, 2H, arom.H1,5), 10.75(s, 2H, D_2O removes, -N$\underline{H}$CO-).

IR (Nujol, ν cm^{-1}): 3380(broad, CH str.), 3300(amide NH str.), 3200, 3120, 3060, 1708(amide C=O str.), 1670(quinone

C=O str.), 1620, 1588(arom. C=C str.), etc.
MS, m/z (rel.intensity): 607([M+1]$^{\cdot+}$,20%), 553(22%), 461([M-2-$C_7H_{15}N_2O$]$^{\cdot+}$,48%), 369(100%), 277(100%), 207 ([$C_{14}H_7O_2$]$^{\cdot+}$,100%), 206([$C_{14}H_6O_2$]$^{\cdot+}$,11%), 171(100%), 143 ([$C_7H_{15}N_2O$]$^{\cdot+}$,75%), 131(50%), 129([$C_6H_{13}N_2O$]$^{\cdot+}$,37%), 93(100%).
Tetra-acetate salt (BSU-1043): mp 235-236°C.

EXAMPLE 15

2,6-Bis(3-(2-hydroxymethyl-1-piperidino)-propionamido)anthracene-9,10-dione (BSU-1040)

2,6-Bis(chloropropionamido)anthracene-9,10-dione (BSU-1013, 6.0 g 14.3 mmol) was suspended in ethanol (300 ml) with stirring and heated to gentle reflux.
2-Hydroxymethylpiperidine (15.18 g, 0.13 mol) in ethanol (100 ml) was added dropwise during 30 mins to the refluxing solution. The mixture was refluxed for 23 hrs, after which time the reaction was judged to have reached completion (TLC). The colour of the reaction mixture was observed to change from mustard yellow to brown during the reflux period. The resulting mixture was cooled using an external ice-water bath and the solid material was collected by filtration. The crude product was washed thoroughly with anhydrous diethyl ether (4 x 100 ml) and dried at 20°C. The product was digested in dimethylformamide (15 ml), treated with activated charcoal and filtered to give a yellow solution. Evaporation of the solvent afforded the title compound as a yellow crystalline solid (6.97 g, 86%th.), m.p. 216-217°C, R_f(EtOH)=0.09.
Anal. ($C_{32}H_{40}N_4O_6.0.5H_2O$):
calcd. C 65.62 H 7.06 N 9.57%,

found C 65.49 H 7.07 N 9.66%.

NMR (DMSO-d_6, δ ppm): 1.1-1.8(m, 12H, $-NCH_2(\underline{CH_2})_3-$), 2.1-2.3(m, 4H, $-N\underline{CH_2}(CH_2)_3-$), 2.5-2.9(AB m, 8H, $-CO\underline{CH_2}\underline{CH_2}N<$), 3.11(m, 2H, $-\underline{CH}CH_2OH$), 3.47(broad d, 4H, $-CH\underline{CH_2}OH$), 4.48(broad s, D_2O removes, 2H, $-CH_2\underline{OH}$), 8.05(dd, J, 2.1 and 8.5 Hz, 2H, arom.H3,7), 8.16(d, J 8.5 Hz, 2H, arom.H4,8), 8.44(d, J, 2.1 Hz, 2H, arom.H1,5), 10.86(s, D_2O removes, 2H, $-N\underline{H}CO-$).

IR (Nujol, ν cm^{-1}): 3440(broad, OH str.), 3300(broad, amide NH str.), 3200, 3120, 3060, 1700(amide C=O str.), 1672 (quinone C=O str.) 1613, 1589(arom. C=C str.), etc.

MS, m/z (rel.intensity): 577($[M+1]^{\cdot+}$,28%), 545($[M-CH_3O]^{\cdot+}$, 8%), 462($[M-C_6H_{12}NO]^{\cdot+}$,2%), 407($[M+1-C_9H_{16}NO_2]^{\cdot+}$,2%), 369 (2%), 277(13%), 185($[C_9H_{17}N_2O]^{\cdot+}$,100%), 168($[C_9H_{16}NO_2-2]^{\cdot+}$, 22%), 128($[C_7H_{14}NO]^{\cdot+}$,100%), 114($[C_6H_{12}NO]^{\cdot+}$,72%).

Diacetate salt (BSU-1044): mp 179-180°C.

EXAMPLE 16

2,6-Bis(3-(N,N-di(2-hydroxyethyl)amino)-propionamido)anthracene-9,10-dione (BSU-1041)

A solution of diethanolamine (25.0 g, 0.24 mol) in EtOH (100 ml) was added dropwise during 30 mins to a stirred, refluxing suspension of 2,6-bis(chloropropionamido)anthracene-9,10-dione (BSU-1013, 8.0 g, 19.1 mmol) in EtOH (150 mL). Reflux was continued for 22 hrs. Removal of volatiles under reduced pressure gave a brown, hygroscopic solid which was digested in 2-propanol (100 ml), triturated with ether (500 mL), and recovered by filtration. After twice repeating this procedure, the solid was washed thor ughly with ether (3 x 100 ml) and dried in vacuo. Recrystallisation from aqueous EtOH (10%

v/v H_2O), with activated charcoal treatment, gave the title compound as a yellow, crystalline solid (9.49 g, 89%), mp 159-160°C.

IR (KBr) 3401br(OH), 3103br(NH), 1685(amide C=O), 1672(quinone C=O) and 1584 cm^{-1}; ^1H-NMR (Me_2SO-d_6) δ 2.86 (t, J = 6.4 Hz, 4H, $CO\underline{CH_2}$), 3.11 (t, J = 4.8 Hz, 8H, $N\underline{CH_2}CH_2OH$), 3.36 (t, J = 6.4 Hz, 4H, $COCH_2\underline{CH_2}$), 3.72 (t, J = 4.8Hz, 8H, $\underline{CH_2}OH$), 5.36 (s, D_2O removes, 4H, OH), 8.06 (dd, J = 2.1 and 8.5 Hz, 2H, H-3,7), 8.18 (d, J = 8.5 Hz, 2H, H-4,8) 8.49 (d, J 2.1 Hz, 2H, H-1,5), 11.00 (s, D_2O removes, 2H, NH); MS, m/z (rel.intensity) 557($[M+1]^{\cdot+}$, 30%), 452($[M-C_4H_{10}NO_2]^{\cdot+}$,12%), 424($[M-C_6H_{14}NO_2]^{\cdot+}$,10%), 397 ($[M+1-C_7H_{14}NO_3]^{\cdot+}$,12%), 223(78%), 185(100%), 157(100%), 152(38%), 139(33%); FAB-MS, m/z (rel.intensity) 557($[M+1]^{\cdot+}$,38%), 429(4%), 277(5%), 215(5%), 185(100%). Anal. ($C_{28}H_{36}N_4O_8$) C,H,N.

EXAMPLE 17

General procedure for the synthesis of acetate salts

The base compounds of formula (I) (4-6 mmol) were suspended in glacial acetic acid (30 ml), treated with activated charcoal (~0.5 g) and then heated to 50-60°C using an external water bath. The resulting brightly-coloured solutions were filtered to remove insoluble material (Whatman No. 1 paper). Trituration with anhydrous diethyl ether afforded bright yellow precipitates. The resulting hygroscopic solids were digested with anhydrous diethyl ether (5 x 100 ml), filtered and dried in vacuo at room temperature (<0.1 mmHg, 48 hrs) to give the acetate salts as amorphous powders.

The elemental analysis of a representative tetra-acetate salt BSU-1025, derived from the free base compound

BSU-1008 (i.e BSU-1008.$(CH_3CO_2H)_4$), is indicated:

Anal. ($C_{36}H_{50}N_6O_{12}$):

calcd. C 56.98 H 6.64 N 11.08%,

found C 56.84 H 6.61 N 11.37%.

EXAMPLE 18

General procedure for the synthesis of quaternary methiodide (methylammonium) iodide salts

The base compounds of formula (I) (2-3 mmol) were suspended in acetone (50-75 ml) with stirring at room temperature. An excess quantity of iodomethane (~10 ml) was added to the mixture and stirred for 24 hrs at room temperature. The resulting quaternary methiodide salt products were collected by filtration, thoroughly washed with anhydrous diethyl ether (3 x 100 ml) and dried at 20°C.

The elemental analysis of a representative diquaternary methylammonium salt BSU-1029, derived from the free base compound BSU-1017 (i.e. BSU-1017.$(CH_3I)_2$), is indicated:

Anal. ($C_{30}H_{42}N_4O_4I_2$):

calcd. C 46.40 H 5.45 N 7.22 I 32.69%,

found C 46.22 H 5.23 N 7.11 I 32.03%.

EXAMPLE 19

This Example illustrates the interaction of compounds of formula (I) and their salts with DNA.

Calf thymus DNA was complexed with the acetate salts tabulated below, in DNA:drug ratios of 40:1, 20:1 and 10:1 respectively. The increase in melting temperature (ΔT_m) for the DNA was then measured at each ratio. The results tabulated in Table 3 are mean values of T_m based on at least three measurements, with esd values of ±(0.1-0.3)°C. The estimated pKa value for each compound is also shown.

TABLE 3

COMPOUND NO.	n	EST. pK_a	T_m (°C) OF CALF THYMUS DNA 40:1	20:1	10:1
MITOXANTRONE	-	-	ND	7.4	≥15.0
BSU-1022	1	~7.4	0.5	0.5	0.5
BSU-1025	1	~9.5, ~6	4.2	8.2	13.6
BSU-1024	1	~10	3.7	7.7	13.5
BSU-1032	1	~10	ND	8.2	14.3
BSU-1028	2	~7.7	ND	3.0	4.9
BSU-1031	2	~9, ~5	ND	4.4	9.2
BSU-1030	2	~10.8	3.7	7.7	≥15.5
BSU-1021	2	~10.4	3.7	7.3	≥15.3
BSU-1043	2	~9.5, ~5	2.9	4.6	7.2[a]
BSU-1041	2	~9	3.2	6.1	10.9[b]
BSU-1044	2	~10	3.8	7.9	14.4
BSU-1037	2	~10	4.1	9.2	≥15.3
BSU-1042	2	~10	4.3	8.3	≥15.0

[a] the T_m of this compound at 5:1 ratio was 11.5±0.1°C
[b] the T_m of this compound at 5:1 ratio was 14.3±0.1°C
ND = not determined.

EXAMPLE 20

This Example further illustrates the interaction of certain compounds of formula (I) and their salts with different DNA's.

Three different types of DNA; Clostridium perfringens, calf thymus and Micrococcus lysodeikticus were each complexed with the acetate salts tabulated below in a DNA:drug ratio of 10:1. The increase in melting temperature (ΔTm) was then measured in each case. The results tabulated in Table 4 are mean values of Tm based on at least three measurements, with esd values of ±(0.1-0.3)°C.

The Table also lists possible binding site preferences. Values obtained for mitoxantrone are included for comparison.

TABLE 4

COMPOUND NO. -NR^1R^2.HOAC	n	ΔT_m (°C) OF DIFFERENT DNAs AT A DNA:DRUG RATIO OF 10:1			DNA BINDING SITE[e] PREFERENCE
		CP[a]	CT[b]	ML[c]	
MITOXANTRONE	-	>26.4	≥15.0	>11.7	AT?
BSU-1024	1	16.6	13.5	>16.6	NONE
BSU-1030	2	≥20.5	≥15.5	>18.1	NONE
BSU-1041	2	11.7	10.9	≥15.7	GC?
BSU-1032	1	19.0	14.3	>18.4	NONE
BSU-1021	2	≥18.9	≥15.3	>14.5	AT?
BSU-1037	2	≥18.5	≥15.3	>14.6	AT?

[a] Clostridium perfringens (CP, 72% A-T, T_m of native was 61.1±0.2°C)
[b] Calf thymus (CT, 58% A-T, T_m of native was 67.0±0.2°C)
[c] Micrococcus lysodeikticus (ML, 28% A-T, T_m of native was 72.0±0.2°C)
[e] Possible binding site preferences

EXAMPLE 21

In this Example, the "critical concentrations" and calculated unwinding angles of cccDNA (PM-2) obtained with acetate salts of compounds of formula (I) were determined.

The "critical concentration" is the concentration of drug at which the closed PM-2 almost co-migrates with nicked DNA. The "unwinding angle" (ϕ) is the degree of local unwinding of the DNA helix due to each molecule of drug bound to DNA with values estimated ±2°. The results are tabulated below in Table 5. Values obtained for ethidium bromide are included for reference.

Together with the results shown in Examples 19 and 20, the results obtained here suggest that intercalation is the major mode of binding of the compounds with DNA.

TABLE 5

COMPOUND NO.	CRITICAL CONCENTRATION[a] (μmol dm^{-3})	UNWINDING ANGLE° (ϕ)[b]
ETHIDIUM BROMIDE	24.9	26
BSU-1025	9.7	17
BSU-1021	4.0	21
BSU-1037	8.7	32
BSU-1030	10.4	26
BSU-1041	24.9	23

EXAMPLE 22

In this Example, in vitro studies were carried out to measure the cytotoxicity of acetate salts and methylammonium iodide salts of compounds of formula (I).

Three different cell lines were used; L1210, WS and V79. In each case the concentration of compound required to kill 50% of the cell population (IC_{50}) was determined.

The results for the acetate salts and the methylammonium salts are shown in Tables 6 and 7 respectively. In each case values for mitoxantrone are shown for comparison.

TABLE 6

COMPOUND NO.	n[a]	pK_a Est.	Ic_{50}[b] VALUES IN DIFFERENT CELL LINES (μmol dm^{-3})		
			L1210	WS	V79
MITOXANTRONE	NA	~7.4	0.002	0.01	ND
BSU-1022	1	>100	>100	>100	
BSU-1025	1	~9.5, ~6	6.50	4.60	4.60
BSU-1024	1	~10	27.0	19.0	18.0
BSU-1032	1	~10	76.0	>100	30.0
BSU-1028	2	~8	0.57	4.20	1.60
BSU-1031	2	~9, ~5	1.70	3.10	1.80
BSU-1030	2	~10.80	0.34	2.70	2.90
BSU-1021	2	~10.4	0.19	2.00	1.70
BSU-1041	2	~9	4.20	>100	25.5
BSU-1044	2	~10	0.50	2.80	3.60
BSU-1037	2	~10	0.28	3.60	12.0
BSU-1042	2	~10	2.30	5.50	3.95
BSU-1043	2	~9.5, ~5	4.20	6.60	19.5

ND = not determined
NA = not applicable

TABLE 7

COMPOUND NO.	n[a]	IC_{50}[b] VALUES IN DIFFERENT CELL LINES (μmol dm^{-3})		
		L1210	WS	V79
MITOXANTRONE -	NA	0.002	0.01	ND
BSU-1003	1	4.70	40.0	2.55
BSU-1005	1	100	>100	31.0
BSU-1007	1	5.60	54.0	26.0
BSU-1026	2	1.00	2.20	2.10
BSU-1027	2	0.48	4.80	3.10
BSU-1029	2	2.20	5.20	2.35

NA = not applicable

EXAMPLE 23

In this Example, in vivo studies were carried out on the L1210 leukaemia model tumour system using the acetate salts BSU-1032, BSU-1021, BSU-1037, BSU-1042, BSU-1043 and BSU-1025 at varying doses.

The salts were administered intraperitoneally to test animals once a day on days 3, 5, 6 and 7 for several weeks.

The percentage life span (%LS) of the test animals was determined in comparison to a control (100%) to which a saline solution was administered. The percentage increase in life span of the animals was also determined.

The results are shown in Table 8 below.

TABLE 8

GROUP	COMPOUND NO.	DOSE mg/kg	% LS OF CONTROL	% ILS
1	SALINE	0	100.0	0
2	BSU-1032	3.125	97.1	-2.9
3	BSU-1032	6.25	93.3	-6.7
4	BSU-1032	12.5	91.5	-8.5
5	BSU-1032	25.0	89.7	-10.3
6	BSU-1032	50.0	93.3	-6.7
7	BSU-1032	100.0	99.0	-1.0
8	BSU-1021	3.125	97.8	-12.2
9	BSU-1021	6.25	97.1	-2.9
10	BSU-1021	12.5	85.9	-14.1
11	BSU-1021	25.0	87.8	-12.2
12	BSU-1021	50.0	987.1	-2.9
13	BSU-1021	100.0	87.8	-12.2
14	BSU-1037	3.125	101.0	1.0
15	BSU-1037	6.25	84.2	-15.8
16	BSU-1037	12.5	90.5	-9.5
17	BSU-1037	25.0	37.8	-62.2
18	BSU-1037	50.0	35.7	-64.3
19	BSU-1037	100.0	31.5	-68.5
20	BSU-1042	12.5	101.0	1.0
21	BSU-1042	25.0	92.6	-7.4
22	BSU-1042	50.0	90.5	-9.5
23	BSU-1042	100	117.8	17.8
24	BSU-1042	200	136.8	36.8
25	BSU-1042	400	130.5	30.5
26	BSU-1043	12.5	111.5	11.5
27	BSU-1043	25.0	117.8	17.8
28	BSU-1043	50.0	120.0	20.0

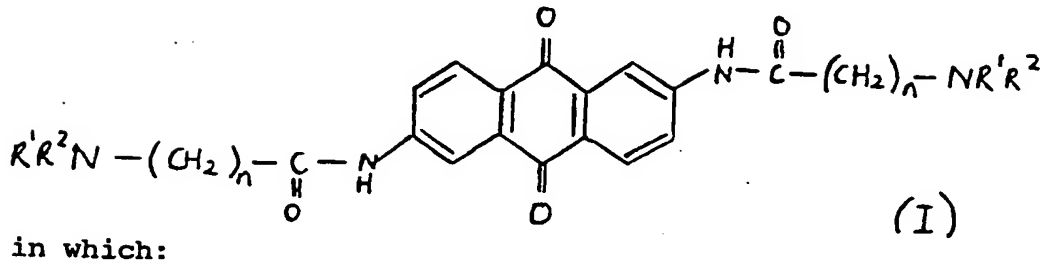
TABLE 8 (cont)

GROUP	COMPOUND NO.	DOSE mg/kg	% LS OF CONTROL	% ILS
29	BSU-1043	100	134.7	34.7
30	BSU-1043	200	86.3	-13.7
31	BSU-1043	400	63.1	-36.9
32	BSU-1025	6.25	103.2	3.2
33	BSU-1025	12.5	96.7	-3.3
34	BSU-1025	25.0	111.8	11.8
35	BSU-1025	50.0	111.8	11.8
36	BSU-1025	100	107.5	7.5
37	bsu-1025	200	32.2	-67.8

[a] Schedule = adminstration once daily, i.p on days 3,5,6 and 7
[b] Percentage life span of test animals in comparison to control (100 %)
[c] Percentage increase in life-span

CLAIMS

1. A compound having the general formula (I):



(I)

in which:

n is 1, 2 or 3; and

R^1 and R^2 are each, independently, an ethyl, hydroxyethyl, or hydroxymethyl group; or R^1 and R^2, together with the nitrogen atom to which they are attached, form a cyclic group which is a 1-piperidino, 2- or 4-(2-hydroxyethyl)-1-piperidino, 2-hydroxymethyl-1-piperidino, 4-(2-hydroxyethyl)- or 4-methyl-1-piperazino, or 4-morpholino group;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 which is:

2,6-bis(2-(1-piperidino)acetamide)anthracene-9,10-dione;

2,6-bis(2-(4-morpholino)acetamido)anthracene-9,10-dione;

2,6-bis(2-diethylaminoacetamido)anthracene-9,10-dione;

2,6-bis(2-(4-methyl-1-piperazino)acetamido)anthracene-9,10-dione;

2,6-bis(3-(1-piperidino)propionamido)anthracene-9,10-dione;

2,6-bis(3-(4-morpholino)propionamido)anthracene-9,10-dione;

2,6-bis(3-diethylaminopropionamido)anthracene-9,10-dione;

2,6-bis(3-(4-methyl-1-piperazino)propionamido)anthracene-9,10-dione;

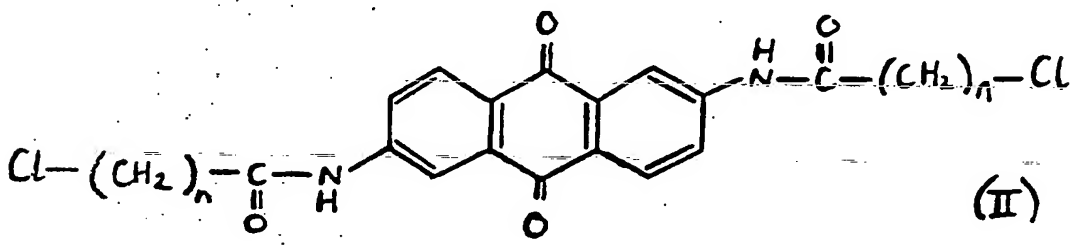
2,6-bis(3-(2-(2-hydroxyethyl)-1-piperidino)-propionamido) anthracene-9,10-dione;

2,6-bis(3-(4-(2-hydroxyethyl)-1-piperidino)-propionamido) anthracene-9,10-dione;

2,6-bis(3-(4-(2-hydroxyethyl)-1-piperazino)-propionamido)

anthracene-9,10-dione;
2,6-bis(3-(2-hydroxymethyl-1-piperidino)-propionamido) anthracene-9,10-dione; or
2,6-bis(3-(N,N-di(2-hydroxyethyl)amino)-propionamido) anthracene-9,10-dione.

3. A process for preparing a compound of formula (I) as claimed in claim 1 which comprises treating a compound of the general formula (II):



(II)

in which n is 1, 2 or 3, in the presence of an organic solvent, with an amine of formula HNR^1R^2 in which R^1 and R^2 are as defined in claim 1.

4. A compound of formula (I) as claimed in claim 1 or 2, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer.

5. Use of a compound of formula (I) as claimed in claim 1 or 2, or a pharmaceutically acceptable salt thereof, in the preparation of a medicament for use in treating cancer.

6. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active principle, a compound as claimed in claim 1 or 2.

7. A method of treating a host suffering from cancer which method comprises administering thereto a pharmaceutically effective amount of a compound of formula (I) as claimed in claim 1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01004

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 C 237/04, C 07 D 295/14, A 61 K 31/33, 31/06, C 07 D 211/20

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 C 237/00, C 07 D 295/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 3859315 (SANTILLI et al.) 7 January 1975 see example 3; claims --	1-3,6
X	Arzneimittel Forschung, volume 29, no. 10, October 1979, E. Winkelmann et al.: "Chemotherapeutically active anthraquinones", pages 1504-1509 see page 1505, table I, compound no. 14 --	1-3,6
X	DE, A, 2702137 (BIBER, Rudolf) 29 September 1977 see claim --	1-8
A	WO, A, 86/00892 (BIBER, Rudolf) 13 February 1986 see the whole document -- ./.	1,6-8

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16th November 1990	10. 12. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nuria TORIBIO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	FR, A, 2321881 (HOECHST) 25 March 1977 see examples; claims --------	1,6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9001004 SA 38229

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/11/90 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		JP-A-	52027759	02-03-77
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